239 ENGINEERING OF POTENCY-ENHANCED TCR-EDITED T CELLS FOR SHARED NEOANTIGEN-TARGETED CANCER IMMUNOTHERAPY

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Background Human cancers can express both private and shared mutation-associated neoantigens, the latter being derived from recurrent cancer driver gene mutations, with the R175H mutation in the human *TP53* tumor-suppressor gene being the most frequently shared between patients across cancer types. This makes the generation and subsequent adoptive transfer of TCR-engineered T cells reactive to an HLA-A*02:01-restricted $TP53^{R175H}$ epitope a highly attractive cancer immunotherapy approach. However, the combined immunosuppressive tumor-microenvironment and the low levels of neoantigen presentation by solid cancers compromise the activation and reactivity of TCR-engineered T cells, posing a major hurdle for these cells to eradicate tumor lesions. A substantial body of work has highlighted that inactivation of negative regulators of TCR signaling can augment T cell functionality and anti-tumor reactivity.

Methods Aiming to develop a highly potent anti-cancer T cell product, we applied CRISPR genome engineering to specifically introduce a $TP53^{R175H}$-specific TCR into the TRAC locus of primary human T cells, while simultaneously inactivating negative regulators of TCR signaling. After validating successful introduction of the $TP53^{R175H}$ TCR and inactivation of endogenous TCR chains and the TCR inhibiting genes of interest, the ability of edited T cells to proliferate, produce cytokines and control tumor growth were assessed in vitro.

Results We found that inactivation of TCR signaling inhibitory genes significantly enhanced the ability of TCR-edited T cells to proliferate and produce the cytokines IFN-γ, TNF and IL-2, both in response to anti-CD3/anti-CD28 stimulation and HLA-A*02:01^{+} tumor cell lines that express the $TP53^{R175H}$ mutation. Notably, T cells that had lost expression of negative regulators of TCR signaling were also superior in serial killing of $TP53^{R175H}$-positive tumor cells in vitro, while these T cells did not inhibit tumor cells that overexpressed a TP53 R175 WT epitope.

Conclusions Taken together, our findings show that inactivation of negative regulators of the TCR signaling pathway can enhance the reactivity of TCR-edited T cells against shared neoantigen-expressing tumor cells, thereby providing a promising new approach to increase the efficacy of TCR therapy directed against solid human cancers.

Ethics Approval Samples were obtained through a supply agreement with Sanquin Bloedvoorziening Amsterdam. These samples were collected only from voluntary, non-remunerated, adult donors who provided written informed consent as part of routine donor selection and blood collection procedures, that were approved by the Ethics Advisory Council of Sanquin Blood Supply Foundation.

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